Animal Reproduction Science 119 (2010) 178–182

Contents lists available at ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

Calving traits of crossbred Brahman cows are associated with Heat Shock Protein 70 genetic polymorphisms

Charles Rosenkrans Jr.[a,*], Andrea Banks[a], Sara Reiter[a], Michael Looper[b]

[a] Department of Animal Science, University of Arkansas, Fayetteville, AR 72701, USA
[b] USDA-ARS, Dale Bumpers Small Farms Research Center, Booneville, AR 72927, USA

ARTICLE INFO

Article history:
Received 9 September 2009
Received in revised form 25 January 2010
Accepted 5 February 2010
Available online 12 February 2010

Keywords:
Cattle
Hsp70
SNP
Promoter
Tall fescue

ABSTRACT

Stress proteins and their genetic polymorphisms have been associated with decreased male and female fertility. Objectives were to: (1) identify single nucleotide polymorphisms (SNP) located in the promoter region of the bovine heat shock protein 70 (Hsp70) gene, and (2) evaluate associations between Hsp70 SNP, Julian calving date, and calving rates of crossbred Brahman cows. Specific primers were designed for PCR amplification of a 539 base segment of the bovine Hsp70 promoter (GenBank accession number M98823). Eleven single nucleotide polymorphisms were detected; one deletion at base position 895 ($n = 37$), seven transitions (G1013A, $n = 2$; G1045A, $n = 8$; C1069T, $n = 4$; A1096G, $n = 14$; G1117A, $n = 12$; T1134C, $n = 7$; and T1204C, $n = 56$), and three transversions (A1125C, $n = 53$; G1128T, $n = 51$; and C1154G, $n = 11$). Cows that were homozygous for the minor allele at transversion site A1125C or G1128T had lesser ($P < 0.05$) calving percentages than cows that were homozygous for the primary allele (48 vs. 75%). Homozygous and heterozygous deletion of cytosine at base 895 resulted in lower ($P < 0.05$) calving percentages than homozygous cytosine cows (8, 50, 82%; respectively). In addition, homozygous deletion cows had the latest ($P < 0.05$) Julian calving date. Eighteen Hsp70 promoter haplotypes were deduced, and seven of those haplotypes ($n = 37$) included the deletion at base 895. Thirty-two cows had the haplotype consistent with the GenBank sequence and the remaining 30 cows had a SNP other than the deletion. Cows with deletion haplotypes had a lesser ($P < 0.05$) calving percentage, and the latest ($P < 0.05$) Julian calving date when compared with cows having other SNP haplotypes. Results from the present study suggest that the promoter region of the bovine Hsp70 gene is polymorphic and may be useful in selecting cows with a greater fertility.

© 2010 Elsevier B.V. All rights reserved.

1. Introduction

Environmental stressors, heat stress in particular, can reduce livestock productivity by billions of dollars annually (St-Pierre et al., 2003). Heat stress can directly reduce cattle efficiency, but it also can exacerbate the detrimental effects of other stressors such as ergot alkaloids commonly found in certain forages (i.e., endophyte-infected tall fescue) on the reproductive axis of cattle (Burke et al., 2001; Looper et al., 2009). One approach to solving the impact of stress on cattle productivity is to establish breeding programs that select animals with tolerance to stress. Breed of cattle was related to differences in heat tolerance and fertility (Turner, 1982). Furthermore, heterosis and breed of cattle have been shown to improve cattle tolerance of environmental stress induced by ergot alkaloids, which confirms a genetic linkage to reproductive fitness (Brown et al., 1997, 2000).

Genetic and physiological mechanisms involved in cattle response to heat stress were recently reviewed (Collier

* Corresponding author. Tel.: +1 479 575 4376; fax: +1 479 575 7294.
E-mail address: crosenkr@uark.edu (C. Rosenkrans Jr.).

0378-4320/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.anireprosci.2010.02.005

et al., 2008). Heat shock protein 70 (Hsp70) is one of the most abundant members of the heat shock protein family (Lindquist and Craig, 1988). The Hsp70 molecule has many functions, one of those functions is to serve as a molecular chaperone after an organism is exposed to pathological or environmental conditions, such as viral or bacterial infection, fever, ischemia, heat shock, heavy metals, amino acid analogs, antibiotics, etc. (Ohtsuka and Hata, 2000). Expression of the Hsp70 gene is, in part, under the control of upstream elements in the promoter region (Wu, 1984). Polymorphisms found in the 5′ flanking region of the Hsp70 gene have been associated with decreased pregnancy percentage and diminished semen quality in swine (Huang et al., 2002). Association of polymorphisms of the Hsp70 gene with fertility in cattle is unknown. Therefore, understanding the genetic mechanisms associated with Hsp70 may lead to genetic tools which will improve cattle tolerance of stressors. Objectives of the present study were to: (1) identify single nucleotide polymorphisms (SNP) located in the promoter region of the bovine Hsp70 gene, and (2) evaluate associations between Hsp70 SNP, Julian calving date, and calving percentage of Brahman-influenced cows.

2. Materials and methods

2.1. Animals and sample collection

Crossbred Brahman-influenced cows ($n = 99$) grazed stockpiled and spring-growth, endophyte-infected tall fescue [*Lolium arundinaceum* (Schreb.) Darbysh] pastures prior to the breeding season. Mean body condition score of cows was 5.4 ± 0.5 (1 = emaciated to 9 = obese; Wagner et al., 1988), and mean body weight was 476 ± 16 kg. During the breeding season cows grazed common bermudagrass [*Cynodon dactylon* (L.) Pers]. The committee for animal welfare at the USDA-ARS, Dale Bumpers Small Farms Research Center, Booneville, AR, approved the animal procedures used in this experiment.

Blood samples were collected from all cows at 35 days before the breeding season was initiated. All blood tubes were placed in ice immediately after collection and transported to the laboratory. Tubes containing EDTA treated blood were cooled to 4 °C, centrifuged ($1500 \times g$ for 25 min.), plasma decanted, and buffy coats harvested and stored at −80 °C until DNA extraction.

2.2. DNA isolation, amplification, and analysis

Genomic DNA was extracted from buffy coats using the QIAamp blood and body fluid spin protocol (QIAGEN, Valencia, CA). A Hoefer DyNA Quant 200 fluorometer (Amersham Biosciences Corp., Piscataway, NJ) was used to quantify DNA following purification.

Based on the bovine Hsp70 gene sequence (GenBank accession number M98823), primers were designed using Primer 3 software (Rozen and Skaletsky, 2000). Specific primers [forward (HSP-Pro749F; 5′-GCCAGGAAACCAGAGACAGA-3′) and reverse (HSP-Pro1268R; 5′-CCTACGCAGGAGTAGGTGGT-3′)], were commercially synthesized (Invitrogen, Carlsbad, CA) and used for DNA amplification via polymerase chain reaction (PCR). A Peltier thermal cycler (MJ Research, Waltham, MA) was used for PCR. Each PCR began with an initial 2-min heating at 94 °C, followed by 35 cycles at 94 °C for 30 s, 1 min at 55 °C, and 1 min at 68 °C. A final elongation step consisted of 10 min at 68 °C. Included in each PCR was 100 ng genomic DNA, 0.20 μM of each primer, and 45 μl of Platinum PCR Supermix (Invitrogen, Carlsbad, CA) for a total volume of 50 μl. Amplicons were visualized by electrophoresis in 1.2% agarose gels stained with ethidium bromide in 1.0 X Tris/Boric Acid/EDTA.

Amplification products were purified using the QIAquick 96 PCR purification kit (Qiagen, Valencia, CA). Purified PCR products were sequenced at the University of Arkansas DNA Core Lab using an ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA). Sequences were compared using the web-based software package ClustalW (http://www.ebi.ac.uk/Tools/clustalw2/index.html; European Bioinformatics Institute, Cambridge, UK).

2.3. Statistical analysis

Julian date of calving was analyzed by one-way ANOVA (SNP served as the main effect, and each SNP site was analyzed in a separate analysis). When *F*-test for main effect was significant ($P < 0.05$), multiple *t*-tests were performed for means separation. Within each SNP, genotype effects on calving percentage were tested by Chi-square. Power analyses were conducted by Java Applets for Power and Sample Size (Lenth, 2006–2009), and the POWER procedure of SAS (SAS Inst., Inc., Cary, NC, USA).

3. Results

3.1. Identification of polymorphisms

A 539-bp segment of the bovine Hsp70 gene promoter region (GenBank accession number M98823 base positions 749–1288) was amplified and sequenced. One deletion, seven transitions, and three transversions were identified (Table 1). The SNP with a minor allele frequency of greater than 10% were selected for additional analyses. Those SNP were C895D [cytosine was deleted and not replaced with a base (D)], A1125C (adenine replaced with cytosine), G1128T (guanine replaced with thymine), and T1204C (thymine replaced with cytosine).

3.2. Base position 895

Deletion of cytosine was detected at base 895. Thirty-seven cows were either heterozygous or homozygous for the deletion (Table 1). Cows that were homozygous deletion had a lesser ($P < 0.01$) calving percentage when compared with heterozygous or homozygous cytosine cows. In addition, heterozygous cows had a lesser ($P < 0.01$) calving percentage than homozygous cytosine cows (Fig. 1). Cows that were homozygous deletion had a later ($P < 0.01$) Julian date of calving when compared with homozygous cytosine cows (Fig. 1).

Table 1
Distribution of SNP in a 539-bp amplicon of the bovine heat shock protein 70 promoter.

Polymorphism[a]	Genotype distribution[b]			MAF[c]
	Homo	Hetero	Homo	
C895D	62	24	13	25.3
G1013A	97	2	0	1.0
G1045A	91	2	6	7.1
C1069T	95	3	1	2.5
A1096G	85	12	2	8.1
G1117A	87	6	6	9.1
A1125C	46	18	35	44.4
G1128T	48	17	34	42.9
T1134C	92	2	5	6.1
C1154G	88	9	2	6.6
T1204C	43	15	41	48.9

[a] Single nucleotide polymorphism occurred at the number indicated. First letter indicates the primary allele and the letter following the digits is the minor allele (D represents deletion of cytosine).
[b] Number of cows that were homozygous for the primary allele (Homo), heterozygous (hetero), and homozygous for the minor allele (homo).
[c] Minor allele frequency expressed as a percent.

3.3. Base position 1125

A transversion from adenine to cytosine was detected at base 1125. Fifty-three cows were either heterozygous or homozygous with the minor allele (Table 1). Cows that were homozygous cytosine had a lesser ($P<0.05$) calving percentage when compared with heterozygous or homozygous adenine cows (Fig. 2). Genotype at A1125C was not ($P>0.4$) associated with Julian date of calving (78, 78, and 74 ± 3.8 days; respectively for AA, AC, and CC). Retrospective power analysis indicated that for a 0.8 power test approximately 900 cows would have been necessary to detect an A1125C effect on Julian date of calving.

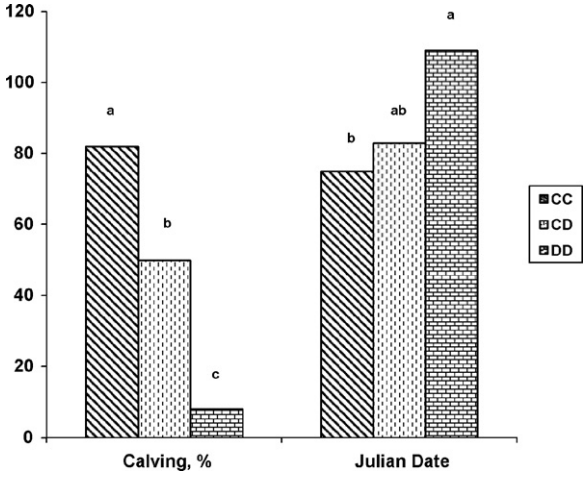

Fig. 1. Association of heat shock protein 70 promoter SNP C895D with percentage of cows calving and Julian date of calving. Genotype distribution for C895D was 62, 24, and 13, respectively for CC, CD, and DD. Pooled SE for Julian calving date was 7.6 days. Percentages and means without a common superscript differ ($P<0.05$).

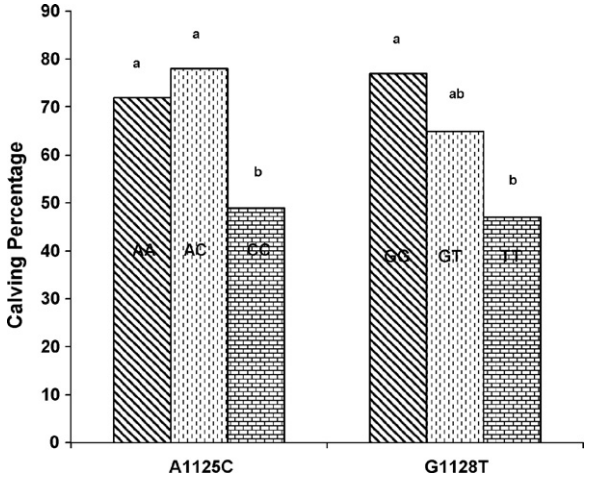

Fig. 2. Association of heat shock protein 70 promoter SNP A1125C and G1128T with percentage of cows calving. Genotype distribution for A1125C was 46, 18, and 35 respectively for AA, AC, and CC. Genotype distribution for G1128T was 48, 17, and 34 respectively for GG, GT, and TT. Percentages within a SNP without a common superscript differ ($P<0.05$).

3.4. Base position 1128

A transversion from guanine to thymine was detected at base 1128. Fifty-one cows were either heterozygous or homozygous with the minor allele (Table 1). Cows that were homozygous thymine had a lesser ($P<0.01$) calving percentage when compared with homozygous guanine cows; however, calving percentages were not different between heterozygous cows and homozygous thymine or homozygous guanine cows (Fig. 2). Genotype at G1128T was not ($P>0.5$) associated with Julian date of calving (76, 82, and 75 ± 3.9 days; respectively for GG, GT, and TT). Retrospective power analysis indicated that for a 0.8 power test approximately 320 cows would have been necessary to detect a G1128T effect on Julian date of calving.

3.5. Base position 1204

A transition from thymine to cytosine was detected at base 1204. Fifty-six cows were either heterozygous or homozygous with the minor allele (Table 1). Genotype at T1204C did not ($P>0.3$) alter calving percentage (65, 80, and 59%), or Julian date of calving (76, 82, and 75 ± 3.8 days; respectively for TT, TC, and CC). Retrospective power analysis indicated that for a 0.8 power test approximately 430 cows would have been necessary to detect a T1204C effect on calving percentage and Julian date of calving.

3.6. Hsp70 promoter haplotypes

Eighteen unique haplotypes were deduced from the 11 SNP sites (Table 2). Cows that were heterozygous for any particular SNP were coded as containing the minor allele. Those haplotypes ranged from 1 to 32 observations. Composite haplotypes were created for subsequent analyses. Haplotype 6 had the same sequence as that published at GenBank; therefore, those cows ($n=32$) with haplotype 6 were categorized as "No SNP". Seven haplotypes (no.

Table 2
Haplotype frequency in a 539-bp amplicon of the bovine heat shock protein 70 promoter.

Haplotype[a]		
Number	Sequence	Observations
1	CAGCAACGTCC	1
2	CAGTAACGTCC	1
3	CGACAAAGCCC	6
4	CGACAGAGTCT	1
5	CGGCAGAGTCC	1
6	CGGCAGAGTCT	32
7	CGGCAGCTTCC	9
8	CGGCGGCTTCG	2
9	CGGCGGCTTCT	1
10	CGGCGGCTTGC	5
11	CGGTAACGTCC	3
12	DGACAACTCCC	1
13	DGGCAGAGTCC	2
14	DGGCAGAGTCT	1
15	DGGCAGATTCT	3
16	DGGCAGCTTCC	19
17	DGGCAGCTTCT	5
18	DGGCGGCTTGC	6

[a] Order of SNP in these haplotypes was C895D, G1013A, G1045A, C1069T, A1096G, G1117A, A1125C, G1128T, T1134C, C1154G, and T1204C; deletion of a cytosine is presented as D; haplotype six represents the published sequence (GenBank accession number M98823).

12–18) represented by 37 cows were categorized as "Deletion", the remaining 30 cows were categorized as "Yes SNP" because they contained some type of SNP other than the deletion at 895.

Composite haplotypes were related to differences in calving percentages, and Julian date of calving. Cows with the deletion had the least ($P < 0.001$) calving percentage (Fig. 3). Furthermore, cows categorized as Deletion had a later ($P < 0.05$) average Julian date for calving when compared with Yes SNP cows (Fig. 3). Retrospective power analysis indicated that for a 0.8 power test approximately 790 cows would have been necessary to detect a difference between No SNP and Yes SNP on calving percentage and Julian date of calving.

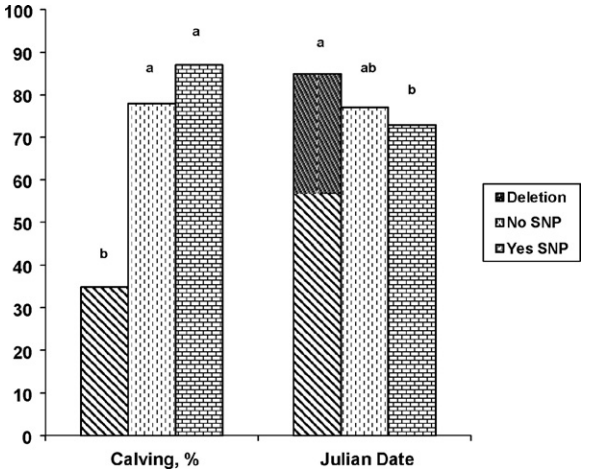

Fig. 3. Association of heat shock protein 70 promoter composite haplotypes with percentage of cows calving and Julian date of calving. Composite haplotype distribution was 37, 32, and 30, respectively for Deletion, No SNP, and Yes SNP. Pooled SE for Julian calving date was 3.6 d. Percentages, and means without a common superscript differ ($P < 0.05$).

4. Discussion

Currently, the bovine genome map is at version 7.1X (Tellam et al., 2009). The intent of this experiment was to add value, via biological relevance, to the sequencing projects, and provide genetic tools for the analysis of cattle fertility. Single nucleotide polymorphisms in the promoter region of the bovine Hsp70 gene (base positions 895, 1125, and 1128) were related to calving percentages. Those findings are consistent with Huang et al. (2002) who found that polymorphisms in the 5′ flanking region of the porcine Hsp70 gene were associated with decreased pregnancy percentages and diminished semen quality in boars.

The deletion of cytosine at base position 895 had the greatest effect on average calving date. Only 8% of cows homozygous with the cytosine deletion calved, and those cows that calved had an average calving date of 109 days which was approximately 35 days longer than cows without the deletion. That polymorphism occurs within 350 bp of the putative transcription start site and is located in a region that has a critical role in heat shock gene induction (for review, see Burdon, 1986; Lindquist and Craig, 1988). Single nucleotide polymorphisms found at base positions 1125 and 1128 appeared to be related. Ninety-seven percent of the cows that were homozygous for the minor allele at 1125 (CC) were homozygous for the minor allele at 1128 (TT). The precise mechanism by which the polymorphisms described herein are associated with calving percentages was not studied; however, control of heat shock protein synthesis has been studied extensively (for review see Collier et al., 2008).

Heat shock protein expression has been detected in gametes and early-stage embryos (Edwards and Hansen, 1996; Sagirkaya et al., 2006; Wilkerson and Sarge, 2009). Those reports suggest that heat shock protein gene expression was related to embryonic survival and overall pregnancy success. Although Hsp70 is induced by stress, circulating concentrations of plasma Hsp70 were found to be repeatable within "non-stressed" cows of various ages (Kristensen et al., 2004). The relationship between maternal serum concentrations of Hsp70 and cellular concentrations of Hsp70 for the dam's postpartum ovary and her maturing oocytes are not known. However, it has been suggested that 60–80 days are required for a bovine follicle to grow from an early preantral stage to a mature stage ready for ovulation, and follicles exposed to adverse conditions during growth could result in impaired oocyte maturation and development (Britt, 1992).

Our working hypothesis is that by understanding the genetic and physiological mechanisms related to cattle reproductive success, we will be able to develop selection tools for increased cattle sustainability and profitability. Analyzing data from multiple SNP sites can be difficult especially with small populations and skewed SNP distributions. Using haplotype models, and composite haplotype models, as used in this experiment, may be useful in developing genetic selection tools. Additional research will be required to determine the physiological mechanisms reg-

ulating the relationships among Hsp70 genotypes, serum and tissue concentrations of Hsp70, and cattle fertility.

5. Conclusions

Genetic markers and/or whole genome sequencing may be useful for improving cattle fecundity. Results of the present study indicate that the upstream elements of the bovine heat shock protein 70 gene are polymorphic. In addition, those polymorphisms were informative with regard to calving percentages of Brahman-influenced cows. Additional research will be needed to establish the physiological mechanisms by which these polymorphisms alter cow fertility and to evaluate genotype by environment interactions.

Acknowledgements

This study was supported in part by USDA-ARS cooperative agreement 58-6227-8-042. The authors gratefully acknowledge L. Huddleston, W. Jackson, D. Jones, G. Robson, S. Tabler, and B. Woolley at USDA-ARS Dale Bumpers Small Farms Research Center for technical assistance and daily animal management. We thank M. Roe, B. Okimoto, R. Okimoto, R. Flores, and L. Starkey for their assistance with DNA analysis and interpretation; and Kathleen Yeater at USDA-ARS-SPA for statistical power analysis.

References

Britt, J.H., 1992. Impacts of early postpartum metabolism on follicular development and fertility. Bovine Pract. 24, 39–43.

Brown, M.A., Brown Jr., A.H., Jackson, W.G., Miesner, J.R., 1997. Genotype X environment interactions in Angus, Brahman, and reciprocal cross cows and their calves grazing common bermudagrass and endophyte-infected tall fescue pastures. J. Anim. Sci. 75, 920–925.

Brown, M.A., Brown Jr., A.H., Jackson, W.G., Miesner, J.R., 2000. Genotype X environment interactions in Angus, Brahman, and reciprocal-cross cows and their calves grazing common bermudagrass, endophyte-infected tall fescue pastures, or both forages. J. Anim. Sci. 78, 546–551.

Burdon, R.H., 1986. Heat shock and the heat shock proteins. Biochem. J. 240, 313–324.

Burke, J.M., Spiers, D.E., Kojima, F.N., Perry, G.A., Salfen, B.E., Wood, S.L., Patterson, D.J., Smith, M.F., Lucy, M.C., Jackson, W.G., Piper, E.L., 2001. Interaction of endophyte-infected fescue and heat stress on ovarian function in the beef heifer. Biol. Reprod. 65, 260–268.

Collier, R.J., Collier, J.L., Rhoads, R.P., Baumgard, L.H., 2008. *Invited Review:* Genes involved in the bovine heat stress response. J. Dairy Sci. 91, 445–454.

Edwards, J.L., Hansen, P.J., 1996. Elevated temperature increases heat shock protein 70 synthesis in bovine two-cell embryos and compromises function of maturing oocytes. Biol. Reprod. 55, 341–346.

Huang, S.Y., Chen, M.Y., Lin, E.C., Tsou, H.L., Kuo, Y.H., Ju, C.C., Lee, W.C., 2002. Effects of single nucleotide polymorphisms in the 5′-flanking region of heat shock protein 70.2 gene on semen quality in boars. Anim. Reprod. Sci. 70, 99–109.

Kristensen, T.N., Løvendahl, P., Berg, P., Loeschcke, V., 2004. Hsp72 is present in plasma from Holstein-Friesian dairy cattle, and the concentration level is repeatable across days and age classes. Cell Stress Chaperones 9, 143–149.

Lenth, R.V., 2006–2009. Java Applets for Power and Sample Size [Computer software]. Retrieved December 12, 2009 from http://www.stat.uiowa.edu/∼rlenth/Power.

Lindquist, S., Craig, E.A., 1988. The heat shock proteins. Annu. Rev. Genet. 22, 631–677.

Looper, M.L., Rorie, R.W., Person, C.N., Lester, T.D., Hallford, D.M., Aiken, G.E., Roberts, C.A., Rottinghaus, G.E., Rosenkrans Jr., C.F., 2009. Influence of toxic endophyte-infected fescue on sperm characteristics and endocrine factors of yearling Brahman-influenced bulls. J. Anim Sci. 87, 1184–1191.

Ohtsuka, K., Hata, M., 2000. Molecular chaperone function of mammalian HSP70 and HSP40—a review. Int. J. Hyperthermia 16, 231–245.

Rozen, S., Skaletsky, H.J., 2000. Primer3 on the WWW for general users and for biologist programmers. In: Krawetz, S., Misener, S. (Eds.). Bioinformatics Methods and Protocols: Methods in Molecular Biology. Humana Press, Totowa, NJ. http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi, pp. 365–386.

Sagirkaya, H., Misirlioglu, M., Kaya, A., First, N.L., Parrish, J.J., Memili, E., 2006. Developmental and molecular correlates of bovine preimplantation embryos. Reproduction 131, 895–904.

St-Pierre, N.R., Cobanov, B., Schnitkey, G., 2003. Economic losses from heat stress by US livestock industries. J. Dairy Sci. 86 (e-Suppl.), e52–e77.

Tellam, R.L., Lemay, D.G., Van Tassell, C.P., Lewin, H.A., Worley, K.C., Elsik, C.G., 2009. Unlocking the bovine genome. BMC Genomics 2009, 10,193 doi:10.1186/1471-2164r-r10-193.

Turner, H.G., 1982. Genetic variation of rectal temperature in cows and its relationship to fertility. Anim. Prod. 35, 401–412.

Wagner, J.J., Lusby, K.S., Oltjen, J.W., Rakestraw, J., Wettemann, R.P., Walters, L.E., 1988. Carcass composition in mature Hereford cows: estimation and effect on daily metabolizable energy requirement during winter. J. Anim. Sci. 66, 603–612.

Wilkerson, D.C., Sarge, K.D., 2009. RNA polymerase II interacts with the Hspa1b promoter in mouse epididymal spermatozoa. Reproduction 137, 923–929.

Wu, C., 1984. Activating protein factor binds in vitro to upstream control sequences in heat shock gene chromatin. Nature 311, 81–84.